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BOVINE GROWTH HORMONE

ABSTRACT OF THE DISCLOSURE

A DNA comprising a deoxynucleotide sequence coding for bovine growth hormone is described. A transfer vector and an expression vector containing this DNA and microorganisms transformed by these vectors are also described.

BACKGROUND OF THE INVENTION

Growth hormone is a polypeptide hormone synthesized in and secreted by the adenohypophysis (anterior lobe of the pituitary). Growth hormone is synthesized as a precursor protein (pre-growth hormone) containing an N-terminal signal peptide and the growth hormone sequence. The amino acid sequence for bovine growth hormone has been determined (Dayhoff, M.D., et al, Atlas of Protein Sequence and Structure, Vol. 5, Supp. 3, pp. 345-352, National Biomedical Research Foundation, Washington, D.C. (1978))

Growth hormone is normally produced throughout life, although in highest amounts during the pre-adult period. The hormone is required for pre-adult growth. Although its mechanism is not understood in detail, growth hormone is known to promote skeletal growth, nitrogen retention, protein synthesis and affects glucose and lipid metabolism. In other words, growth hormone is a general anabolic agent.

Uses of bovine growth hormone are based on its known biological activity described above. Bovine growth hormone may

be administered to young cattle in order to increase their rate
of growth and weight gain, thereby decreasing the time required
between birth and marketing for beef. The resulting increase
in meat production could be significant. Furthermore, bovine
growth hormone differs from ovine growth hormone by only a few
amino acids. Thus, bovine growth hormone may be administered
to sheep to accomplish the same goal in sheep as in cattle,
i.e., increasing rate of growth and weight gain and thus in-
creasing meat production. It is also possible that bovine
growth hormone can be administered to hogs (or other animals) to accomplish the
same goals.

Basic techniques for cloning DNA sequences are now
known. For example, Seeburg, P.H., et al, Nature, 270, 486
(1977) describes the cloning of the rat growth hormone gene;
Shine, J., et al, Nature, 270, 494 (1977) describes the cloning
of the human chorionic somatomammotropin gene; and Derynck, R.,
et al, Nature, 285, 542 (1980) describes the cloning of the
human fibroblast interferon gene.

Methods for the expression of heterologous DNA in a
microorganism are now known. In principle, the heterologous DNA
coding sequence is inserted in a DNA transfer vector at a point
located within an expressible operon. For the production of a hybrid protein The inserted sequence
must be in reading frame phase with the coding sequence of
the operon, and oriented in the same direction with respect
to translation. When the conditions are met, translation of
the operon results in "readthrough" to the inserted coding
sequence such that the protein produced is a fusion protein
comprising an N-terminal amino acid sequence coded by the
expressible operon, followed by an amino acid sequence coded

by the insert. See Polisky, B., et al, Proc.Nat.Acad.Sci.USA, 73, 3900 (1976); Itakura, K., et al, Science, 198, 1056 (1979). Several expressible operons have been employed, including ~~inser-~~ ~~tion in the~~ β-galactosidase, ~~gene, the~~ β-lactamase ~~gene~~ and ~~the~~ tryptophan ~~operon.~~

Abbreviations used herein are those abbreviations commonly accepted and used by one of ordinary skill in the art. For example, these abbreviations are accepted by the J.Biol. Chem., without further elucidation.

SUMMARY OF THE INVENTION

The present invention discloses the cloning of a DNA coding for bovine growth hormone and the expression of the cloned DNA in microorganisms.

mRNA coding for bovine growth hormone is isolated from bovine pituitaries. A reverse transcript (a cDNA copy) of the mRNA is prepared and inserted into a transfer vector. The transfer vector is used to transform bacteria which express the cloned cDNA.

DETAILED DESCRIPTION OF THE INVENTION

A DNA sequence coding for bovine growth hormone is obtained by using the cDNA method. The basic techniques of the cDNA method are known and can be illustrated by Seeburg, P.H., et al, supra, and Derynck, R., et al, supra. The cDNA is synthesized by using an RNA preparation extracted from bovine pituitaries as the template.

The RNA is isolated from bovine pituitaries using conventional techniques. Polyadenylated RNA is isolated by affinity chromatography. The integrity of the polyadenylated

RNA is assessed by performing cell-free translation of the poly-adenylated RNA as described by Miller, W. L. and McCarthy, B. J., J.Biol.Chem., 254, 742 (1979) and Martial, J.A., et al, Proc. Nat.Acad.Sci.USA, 74, 1816 (1977) and analyzing the proteins produced by SDS-acrylamide gel electrophoresis as described by Laemmli, U.K., Nature, 227, 680 (1970). Bovine growth hormone is further identified by an immune precipitation reaction as described by Martial, J.A., et al, Proc.Nat.Acad.Sci.USA, supra.

The polyadenylated RNA is used as the template for preparing a double-stranded cDNA copy using conventional tech-niques. The first cDNA strand is synthesized using reverse transcriptase, an oligo-dT primer and the polyadenylated RNA as described by Miller and McCarthy, supra, and Monahan, J.J., et al, Biochem., 15, 223 (1976). RNA is removed by alkali digestion and the single-stranded cDNA is used to self-prime the synthesis of the second strand by reverse transcriptase. The single-stranded hairpin loop is removed by digestion with S1 nuclease ~~as described by~~ (Leong, J.A., et al, J.Virol., 9, 891 (1972)) as described by Ullrich, A, et al Science 196, 1313 (1977).

The cDNA is now ready for insertion into an appropriate transfer vector using conventional techniques. For example, a synthetic nucleotide containing a recognition site for a par-ticular restriction endonuclease can be blunt-end ligated to the cDNA, The cDNA and transfer vector are separately incu-bated with the restriction endonuclease and then annealed to form the transfer vector containing the cDNA. Alternatively, the cDNA can be dC-tailed using dCTP and terminal transferase as described by Roychoudhury, R., et al, Nucl.Acids Res., 3, 863 (1976). The transfer vector, after digestion with a

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(such as Pst I)

restriction endonuclease∧ can be dG tailed using the same

procedure. The dG-tailed transfer vector and dC-tailed cDNA

are then annealed to form the transfer vector containing

the cDNA. ~~The latter procedure is preferred~~. The transfer

vector containing the cDNA is then used to transform a suitable

host, such as E. coli χ1776, as described by Cooke, N.E., et

al, J.Biol.Chem., 255, 6502 (1980). Colonies are selected for

tetracycline resistance. Selected colonies are screened by

conventional techniques. These may include (1) removing the

cDNA by an appropriate restriction endonuclease and analyzing

it by electrophoresis and hybridization (Southern, E.M., J.Mol.

Biol., 98, 503 (1975)), or (2) replica-plating as described

by Grunstein, M. and Hogness, D.S., Proc.Nat.Acad.Sci.USA, 72,

3961 (1975) and hybridizing with an appropriate probe, or (3) examine

colonies directly for expression by RIA or other techniques.

DNA coding for bovine growth hormone can be prepared

from the insert coding for bovine growth pre-hormone. The DNA

coding for the pre-hormone is removed by an appropriate restric-

tion endonuclease. For example, if the cDNA is inserted in

the Pst I site of the plasmid pBR322, the cDNA insert can be

(purified) (as the cDNA for bovine GH contains two internal Pst I sites)

removed by∧digestion with Pst I∧. The cDNA is then digested

with Hae II. Alternatively, the recombinant plasmid derived

from pBR322 can be digested with Hae II to remove a portion of

(DNA coding for the)

the cDNA insert. The Hae II digestion removes the N-terminal

signal peptide and two of the three bases coding for the N-

terminal Ala of growth hormone. These bases are replaced by

serially incubating the insert with the Klenow fragment of DNA

polymerase I and the appropriate deoxynucleotide, as described by

The cDNA coding for growth hormone is then inserted into an
appropriate transfer vector as described above.

The cloned DNA is expressed in bacteria to yield
either a fusion protein comprising the bovine growth hormone
coded by the inserted sequence, or the bovine growth hormone
itself. Several possible techniques are available as options,
and may include (a) modification of the coding sequences to
provide an exact desired translational starting point; (b)
selection or construction of an optimal expression vector;
(c) post-translational processing, either by exploiting in vivo
processing activity of the host or by in vitro chemical means; a w
direct expression
When a fusion protein is expressed, modification of the cloned
nucleotide sequence will generally be unnecessary as long as
the resulting sequence permits translation of the insert in the
correct reading frame and no stop codons intervene before
the initial codon of the inserted sequence.

Growth pre-hormone or growth hormone is expressed as
a fusion protein by insertion of the cDNA into appropriate sites
within expressed operons (expression vectors) including for
example, the Pst I site in the β-lactamase gene of pBR322
(Villa-Komaroff, L., et al, Proc.Nat.Acad.Sci.USA, 75, 3727
(Seeburg, P., et al Nature 274:795 (1978))
(1978)) the EcoRI site of pBR322 carrying the lac control
region and coding sequence for β-galactosidase (Itakura, K.,
supra), or the HindIII site of the trpD gene of plasmid ptrpED50
(Martial, J., et al, Science, 205, 602 (1979)). Modifications of sequence
length by one or two nucleotides in order to achieve correct
reading frame phase are well known in the art. Insertions
at the Pst I site of pBR322, with the aid of the tailing pro-
cedure, occur in correct phase and reading frame with a probabi-
lity of 1/6.

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Growth pre-hormone or growth hormone is prepared from
a fusion protein susceptible of specific cleavage in vitro.
The cloned nucleotide sequence is modified to code for amino
acid sequences providing specificity for a proteolytic enzyme.
A useful sequence is AspAspAspAspLys, cleaved preferentially
by the enzyme enterokinase, as described in copending applica-
tion Serial No. 125,878, filed February 29, 1980, incorporated
herein by reference. As described therein, a linking nucleotide
sequence coding for the foregoing amino acid sequence is inser-
ted adjacent the nucleotide sequence coding for the amino
terminus of growth pre-hormone.

For growth pre-hormone such insertion requires modi-
fication of the original cDNA insert, by removal of nucleotides
on the 5' end of the growth pre-hormone coding sequence. The
cDNA insert for growth hormone, described supra, does not need to
be modified. The modification of the insert for growth pre-
hormone is accomplished either by controlled digestion of the 3'
end of the insert using 3' exonuclease or T4 DNA polymerase
or by the combination of restriction endonuclease cleavage at
a point to the 5' side of the desired starting point and
chemical synthesis to restore that portion of the desired
sequence thus removed. For further details of these procedures,
see copending application Serial No. 125,878. By following
these procedures, preferably using T4 DNA polymerase and S1
nuclease, the cDNA sequence coding for growth pre-hormone and
lacking the 5' untranslated region is obtained. The linker
nucleotide sequence coding for the foregoing amino acid
sequence is blunt-end ligated to the cDNA coding for either
growth pre-hormone or growth hormone using DNA ligase as
described by Valenzuela et al, Nature, 280, 815 (1979). The

modified cDNA sequence is inserted into a fusion protein ex-
pression vector as previously described. Host bacteria, (such as) E. coli
HB101, or E. coli χ1776, (R21) (or other bacteria) are transformed by the recombinant
vectors bearing the inserted growth pre-hormone coding region.
Transformants are selected for resistance to ampicillin.
Transformants are then grown under conditions suitable for
expression of the fusion protein. After expression of the fusion
protein, the growth pre-hormone or growth hormone is cleaved
out by enzymatic hydrolysis using enterokinase.

By the use of appropriate expression transfer vectors,
the growth pre-hormone of the present invention is expressed
directly, i.e., not fused to any procaryotic protein. The
underlying principle of direct expression is that the inserted
DNA segment entirely replaces the coding segment normally
transcribed and translated by the bacterial control region.
The essential component of the control region to be preserved
is termed the expression unit, which includes a promoter and a
ribosomal binding site capable of acting in the host organism.
It is not necessary to remove all of the nucleotides coding for
the host portion of the fusion protein. The relationship
between the ribosomal binding site and the start codon (AUG)
is such that the start codon may be located anywhere within
3-11 nucleotides of the ribosomal binding site. Shine, J., et
al, Proc.Nat.Acad.Sci.USA, 71, 1342 (1974) and Steitz, J.,
Proc.Nat.Acad.Sci.USA, 72, 4734 (1975). In this 3-11 nucleo-
tide region, the first AUG to be encountered sets the reading
frame for translation. In the case of ptrpE30, derived from
ptrpED50 described, supra, and containing the operator, promoter,
attenuator and ribosome binding sequences of the tryptophan

operon together with the nucleotide sequence coding for seven

amino acids of the trp E portein followed by a HindIII site,

the removal of a minimum of 23-29 nucleotides from the HindIII

site provides a site for insertion of the cDNA insert under

tryptophan operon control.

For the direct expression of growth pre-hormone, the

original cDNA insert is modified as described above to remove

the 5' untranslated region. A vector for direct expression can

be constructed by modification of ptrpE30 by removing 23-29

nucleotides using T4 DNA polymerase and S1 nuclease as described

above. A linker nucleotide sequence containing the restriction

sequence for Bam HI endonuclease is blunt-end ligated to both

the modified cDNA insert and the modified ptrpE30 by the pro-

cedure of Valenzuela, et al, supra. This is done to facilitate

insertion which is performed essentially as described by Ull-

rich, A., et al, Science, 196, 1313 (1977). Host bacteria E.

coli HB101 or B. coli χ1776 are transformed by the recombinant

vectors bearing the inserted growth pre-hormone coding region.

Transformants are selected for resistance to ampicillin and

then grown under conditions suitable for expression of growth

pre-hormone.

Growth hormone can be expressed directly by following

the procedure described in Goeddel, D.V., et al, Nature, 281,

544 (1979). Alternatively, a linker nucleotide sequence con-

taining the Bam HI site and the start codon (AUG) can be blunt-

end ligated to the cDNA coding for growth hormone. This modified

cDNA is then inserted into the modified ptrpE30 as described

above.

Growth pre-hormone is converted to growth hormone

by removal of the N-terminal sequence of hydrophobic amino acids that comprise the signal peptide. In *vitro* removal of the signal peptide ~~is~~ (might be) carried out by treating the protein extracted from transformed, induced cells with a preparation of "rough" microsomes as described by Jackson, R.C. and Blobel, G., *Proc*. *Nat.Acad.Sci.USA*, 74, 5598 (1977). In *vivo* removal of the signal peptide may occur during direct bacterial expression of the growth pre-hormone coding sequence. Bacterial and mammalian signal peptides share sequence similarities. Proteins having mammalian signal peptides may be processed by bacterial cells resulting in excretion of growth hormone into the periplasmic space or into the medium.

Growth pre-hormone and growth hormone synthesized as described are purified by techniques well known in the art, including for example, gel filtration, ion exchange chromatography, affinity chromatography and differential solubility techniques.

The details of the present invention will be further described by the following examples. In these examples, digestions with restriction endonucleases were carried out under conditions optimized for each enzyme. Restriction endonucleases, their nomenclature and site specificity have been described in detail by Roberts, R., *Crit.Rev.Biochem*., 4, 123 (1976). Enzymes were obtained commercially (New England Biolabs, Cambridge, Massachusetts) and optimal conditions according to supplier's recommendations were employed unless noted otherwise. Reverse transcriptase was provided by Dr. J. Beard, Life Sciences, Inc., St. Petersburg, Florida. The use of reverse transcriptase and suitable reaction conditions have been described previously by Seeburg, P.H., et al, *Nature* 276, 795 (1978); Seeburg, P.H.,

et al, *supra*; and Shine, J., et al, *supra*. T4 DNA polymerase was obtained from New England Biolabs. The use of T4 DNA polymerase and suitable reaction conditions have been previously described in copending application Serial No. 125,878. Micrococcal S1 nuclease was obtained from Miles Laboratories, Elkhart, Indiana. The use of S1 nuclease and suitable reaction conditions have been previously described by Ullrich, A., *supra*. Terminal deoxynucleotide transferase was obtained from Enzo Biochemicals, New York, New York. The use of this enzyme and suitable reaction conditions have been previously described by Roychoudhury et al, *supra*. The Klenow fragment of DNA polymerase I was obtained from Boehringer Biochemicals, Indianapolis, Indiana.

EXAMPLE 1

Synthesis of bovine growth pre-hormone cDNA. Female bovine pituitaries were collected shortly after killing and were frozen immediately in liquid nitrogen. Total RNA was prepared by homogenizing the pituitaries in a guanidine thiocyanate solution, Chirgwin, J.M., et al, Biochem., 18, 5294 (1979). The RNA was centrifuged through 5.7M CsCl as described by Ullrich, A., et al, *supra*. The RNA was then extracted with phenol and precipitated with ethanol. Polyadenylated RNA was purified using oligo-dT-cellulose affinity chromatography as described by Miller and McCarthy, *supra*, and Aviv, H. and Leder, P., Proc.Nat.Acad.Sci.USA, 69, 1408 (1972).

The polyadenylated RNA was translated in a cell-free system using rabbit reticulocytes as described by Miller and McCarthy, *supra*, and Martial, J.A., et al, *supra*, (1977). Bovine growt

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pre-hormone synthesized in this sytem was immune precipitated using a heterologous anti-ovine growth hormone antiserum and prepared by adsorption to formalin-fixed Staphylococcus aureus Cowan strain I as described by Martial, J.A., et al, supra, (1977). The ^{35}S-proteins were electrophoresed on 12.5% SDS slab poly-acrylamide gels as described by Laemmli, supra. This analysis indicated that polyadenylated RNA coding for bovine growth pre-hormone represented about 12.6% of the total pituitary polyadenylated RNA.

Polyadenylated RNA was reverse-transcribed into single-stranded cDNA using reverse transcriptase by the pro-cedure described by Miller and McCarthy, supra, and Monahan et al, supra. RNA was removed by alkaline hydrolysis. The single-stranded cDNA was extracted with phenol, chromatographed over G-50 Sephadex (trademark Pharmacia, Inc., Uppsala, Sweden) and ethanol precipitated. The single-stranded cDNA was used to self-prime the synthesis of the second strand of cDNA using reverse transcriptase as described above. The single-stranded "hairpin loop" at the 5' end of the first strand of cDNA was removed by digestion with S1 nuclease as described by Leong et al, supra. The double-stranded cDNA was purified by phenol extraction, chromatography over G-50 Sephadex and ethanol precipitation. The double-stranded cDNA was 3'dCMP tailed using dCTP and terminal transferase as described by Roychoudhury et al, supra.

Plasmid pBR322 was cleaved by Pst I endonuclease and tailed with dGMP by the previously described tailing procedure except that dGTP is used instead of dCTP. 50 ng of the dG-tailed, Pst I cleaved plasmid pBR322 and 20 ng of the dC-tailed

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double-stranded cDNA were annealed in a 50 µl reaction as described by Cooke, et al, _supra_.

Transformation of E. coli χ1776 with the plasmid preparation was carried out as follows. E. coli χ1776 were rendered permeable to DNA by incubation in 75mM $CaCl_2$, 5mM $MgCl_2$, 10mM Tris, pH 7.5, for 20 minutes at 4°C. Plasmid and bacteria were incubated for 60 minutes at 4°C, and then 2 minutes at 41°C. Transformed colonies were selected for tetracycline resistance. The presence of cloned DNA was determined by colony hybridization to freshly prepared bovine pituitary ^{32}P labelled cDNA as described by Grunstein and Hogness, _supra_. Plasmid DNA was prepared from selected colonies, cut with Pst I, electrophoresed on 1% agarose, stained with ethidium bromide and photographed and finally transferred to nitrocellulose filters by the method of Southern, _supra_. The presence of growth hormone sequences in the transferred DNA was assessed by hybridization to cloned full length rat hormone cDNA (Seeburg, P. H., et al, _supra_), labelled by nick translation (Maniatis, R., et al, Proc.Nat.Acad.Sci.USA, 72, 1184 (1975)). One clone was obtained which hybridized with the nick-translated DNA and was designated pBP348. The insert contains 831 base pairs

EXAMPLE 2

Sequence analysis of the cDNA. Plasmid pBP348 was cut with Pst I and the phosphate on the 5' ends of the DNA fragments was removed with alkaline phosphatase and replaced with $[^{32}P]$ phosphate using polynucleotide kinase. Subsequent cutting with a variety of other restriction endonucleases, polyacrylamide gel electrophoresis, and staining and autoradiography of the

bands of DNA provide a restriction map of the cloned DNA. A

large batch of pBP348 was then prepared and cut with Pst I,

Pvu II or Sau 3A, labeled with [γ^{32}P]dATP and polynucleotide

kinase, and then cut with other enzymes to yield DNA fragments

labeled at a single end. These fragments were prepared by

elution from polyacrylamide gel and sequenced as described by

Cooke et al, supra, and Maxam, A.M. and Gilbert, W., Proc.Nat.

Acad. Sci.USA, 74, 1560 (1977). The sequence for the inserted

cDNA is shown in Figure 1 together with the corresponding

predicted amino acid sequence coded by the sense strand, i.e.,

the strand corresponding in sequence to the respective mRNA.

The correct reading frame is recognized by the lack of

termination codons over a substantial portion of the inserts.

The amino acid positions are numbered beginning with the amino-

terminal amino acid of bovine growth hormone and proceeding in

the positive direction to the carboxy terminal end and in the

negative direction to the first AUG codon presumed to be the

point of translation initiation. The sequences suggest, in

common with many other hormones, the synthesis of growth hormone

involves posttranslational processing. The translation of growth

hormone mRNA yields a precursor, growth pre-hormone, containing

a signal peptide which may be released during the transit into

the endoplasmic reticulum.

EXAMPLE 3

Synthesis of cDNA coding for bovine growth hormone.
Plasmid pBP348 is digested with Hae II endonuclease generating

a 1600 base pair fragment. One Hae II site is within the cDNA

insert coding for growth pre-hormone and the second site is

within the pBR322 portion of the plasmid. The digestion yields
the following:

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                        +1   +2

            5' -       C TTC .... 3'

            3' - G CGG AAG .... 5'
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Bovine GH may begin with either the +1 ala or the +2 phe residue
at the NH$_2$ terminal end (Dayhoff et al, _supra_), thus one might
attempt to complete the ala codon or one might attempt to delete
it. This deletion approach is accomplished by incubating the DNA
with dATP and the Klenow fragment of DNA polymerase I, as the first
base of the +2 phe codon (on the 3'→75' strand) is A. This reactio
yields the following:

```
                    +1   +2

            5' - C TTC .... 3'

            3' -   AAG .... 5'
```

The DNA is extracted with phenol and precipitated. Excess
dATP and the digested bases are removed by chromatography on G-50
Sephadex. The remaining 5' protuding C of the +1 ala codon is
then removed with S$_1$ nuclease as described by Shine et al Nature
285:456 (1980). This digestion yields:

```
                    +2

            5' -       TTC .... 3'

            3' -       AAG .... 5'
```

This DNA can be inserted into a transfer vector as described in
Example 1.

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EXAMPLE 4

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Expression of bovine growth hormone. Bovine growth
hormone can be expressed by any of the methods described above.
For purposes of illustration only, production of human growth
hormone by direct expression will be described. It is under-
stood that bovine growth hormone can be prepared in the same
manner and that bovine or human growth hormone can be prepared

by any of the other described methods.

(A)   Expression of bovine growth pre-hormone as a
fusion protein was demonstrated by performing a radioimmunoassay
experiment and performing a minicell experiment.   In the radio-
immunoassay experiment, E. coli χ1776 containing pBR348 or E.
coli containing pBR322 as the control were grown in nutrient
broth and collected by centrifugation.   The cells were resuspen-
ded and lysed and radiolabelled antibody to bovine growth hormone
was added.   The immune complex was precipitated and radioactivity
measured.   This experiment shows that the fusion protein retains
some bovine growth hormone immunoactivity.   To further illus-
trade the production of a fusion protein, a minicell experiment
was performed by following the procedure described by Meagher,
R.B., et al, Cell, 10, 521 (1977).   Figure 2 shows the gel
electrophoresis bands resulting from E. coli χ1776 minicells.
Band (a) was obtained from χ1776 transformed with pBP348.   Band
(b) was obtained from χ1776 transformed with pBR322.   Band (c)
is molecular weight markers.   (A) indicates the fusion product
of β-lactamase and bovine growth pre-hormone.   (B) indicates
pre-lactamase and (C) indicates β-lactamase.   This experiment
shows that pBP348 makes a fusion protein consisting of 183
amino acids of β-lactamase, 217 amino acids of bovine growth
pre-hormone and a few linking amino acids coded by the normally
untranslated 5' region.   The total molecular weight seen,
approximately 45,000, agrees with the predicted molecular weight
of the hybrid protein.

(B)   For the direct expression of bovine growth pre-
hormone the insert DNA is first separated from pBP348 by Pst I
endonuclease digestion and purified by preparative gel electro-

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phoresis. A 15 µg sample of purified insert DNA is then
modified by suspending the DNA in water to which is added a
concentrated solution of salts such that the final composition
comprises 70mM Tris, pH 8.8, 70mM $MgCl_2$, 10mM dithiothreitol
and 13.75 units of T4 DNA polymerase in a total volume of 250
µl. The reaction mixture is incubated at 37°C for several
minutes and then dATP is added to a concentration of 50mM to
terminate endonucleolytic digestion at the next adenine
residue. After 30 seconds of additional incubation, the
enzyme is inactivated by heat treatment at 65°C for
five minutes. This process is repeated twice more, once
in which dCTP is used in place of dATP and finally in which
dTTP is again used. The treated DNA is recovered by ethanol
precipitation. Digestion with S1 nuclease to provide blunt
ends is carried out as described by Ullrich, A., et al, supra.
This procedure is designed to produce a DNA molecule terminated
at the start codon at the position number -26. Such molecules
will be translated when inserted in an expression vector having
an insertion site about 3-11 nucleotides from the ribosome
binding site sequence of an expression unit.

A vector for direct expression is constructed by modi-
fication of the plasmid ptrpE30 by the removal of 23-29 nucleo-
tides using T4 DNA polymerase and S1 nuclease as described above.

The modified cDNA and the modified expression vector
are provided with a specific linker having the sequence
5'-CCGGATCCGG-3' on one strand and its complementary sequence
on the other by blunt-end ligation using DNA ligase as described
by Valenzuela, supra. The linkers provide restriction sites
sensitive to Bam HI endonuclease which are employed to facili-
tate insertion. Insertion is accomplished by following the
procedure of Ullrich, A., et al, supra. Host bacteria E. coli
HB101 or E. coli χ1776 are transformed by the recombinant vectors

bearing the inserted modified growth pre-hormone coding region and transformants are selected for resistance to ampicillin. A single transformant designated ptrpE30/bGH is selected for further analysis.

Bacterial cells transformed by ptrpE30/bGH are grown in a standard minimal medium (M9) supplemented with Leu, Pro, vitamin B1 and ampicillin at 37°C. In early log phase, the trp operon is induced by the addition of β-indolylacrylic acid (30 μg/ml medium). Control cultures are left uninduced. After three more hours of growth, 1.5 ml of cells are radioactively labelled by the addition of 20 μCi of ^{35}S-L-Met and incubated for 10 minutes. The cells are collected by centrifugation, washed and resuspended in 250 μl of buffer containing 10% (v/v) glycol, 5% (v/v) β-mercaptoethanol and 2.3% (w/v) SDS in 0.0625M Tris, pH 6.8. The suspension is boiled for five minutes, then applied to a 10% (w/v) SDS-polyacrylamide gel and fractionated by electrophoresis. The protein bands are visualized by autoradiography. The results show the existence of a new protein band of about 24,000 daltons not observed in the un-induced or non-transformed cultures.

The bovine growth pre-hormone is purified by conventional techniques including, for example, gel filtration, ion exchange chromatography, affinity chromatography and differential solubility techniques. Growth pre-hormone is converted to growth hormone by following the procedure described by Jackson, et al, supra.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is

intended to cover any variations, uses or adaptations of the
invention following, in general, the principles of the inven-
tion and including such departures from the present disclosure
as come within known and customary practice within the art to
which the invention pertains.